

Recombinant prolylcarboxypeptidase activates plasma prekallikrein

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Recently, we recognized that the serine protease prolylcarboxypeptidase (PRCP), an angiotensin II degrading enzyme, isolated from HUVEC is a plasma prekallikrein (PK) activator. These data indicate that there is a physiologic basis for activation of the proteins of the plasma kallikrein/kinin system (KKS) when assembled on HUVEC membranes. Present investigations determined if recombinant PRCP (rPRCP) activates PK. PRCP cDNA was cloned in pMT/BIP/V5-HIS-C followed by transient transfection into S2 Schneider insect cells and purified from serum-free media. Mature rPRCP, but not that which has the propeptide, activated PK when bound to high molecular weight kininogen (HK) linked to microtiter plates. Recombinant PRCP's ability to activate PK was inhibited by antipain (IC₅₀ = 3 μM), leupeptin (IC₅₀ = 3 μM), KPI domain of the amyloid precursor protein (IC₅₀ = 60 μM), and corn trypsin inhibitor (IC₅₀ = 10 nM), but not by 1-3 mM EDTA, o-phenanthroline, iodoacetamide, or iodoacetic acid. Angiotensin II or bradykinin (BK), but not angiotensin II (1-7) or BK (1-5), inhibited PK activation with an IC₅₀ = 0.2 or 0.1 mM, respectively. These findings are similar to PRCP isolated from HUVEC. Anti-PRCP inhibited rPRCP's ability to activate PK. Unlike factor XIIa, rPRCP's ability to activate PK is blocked by angiotensin II but not neutralizing antibody to factor XIIa. The K_m of rPRCP activation of PK was 17 nM. PRCP antisense oligonucleotide inhibited PRCP activity on HUVEC, indicating that PRCP is the major PK activating enzyme. On flow cytometry, PRCP is specifically found on the membrane of nonpermeabilized HUVEC in suspension. On laser scanning confocal microscopy, both PRCP and LAMP1 are on the membrane of nonpermeabilized cells. When permeabilized, PRCP and LAMP1 antigen do not co-localize in lysosomes. On the membrane of fixed but not permeabilized HUVEC, PRCP co-localizes with all of the HK binding proteins in the following order: gC1qR, uPAR, and cytokeratin 1. PRCP appears to be the major PK activator on HUVEC. These data support the hypothesis that the KKS counterbalances the renin-angiotensin system. The interaction of these systems may serve as a direct link between blood pressure control and thrombosis *in vivo*.

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